# AN INVESTIGATION OF THE POLYSACCHARIDES PRESENT IN SUGAR MILL SYRUPS

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*Summary*

Two major polysaccharides have been found in syrups obtained from various sources. They were separated by fractional precipitation with ethanol, and the homogeneity of the fractions was checked by moving-boundary electrophoresis. One of the polysaccharides was found in all samples examined and appeared to be a hemicellulose type. The other polysaccharide was a polyglucose of the dextran type, and its appearance was associated with large increases in the viscosity of the syrup. The possible origins of the polysaccharides are discussed.

## I. INTRODUCTION

The isolation and identification of a dextran polysaccharide found in sugar-cane was the subject of a previous report from these Laboratories (Nicholson and Lilienthal 1959). Data gathered at that time showed that there was a variation in the concentration of this polysaccharide during the cane-crushing season. A check on the viscosity of sugar syrups prepared from the cane by crushing, clarification of the juice, and subsequent concentration showed that large increases in the viscosity of syrups occurred at certain times during the sugar-milling season. Since water-soluble polysaccharides present in the sugar-cane should carry through into the syrup, certain syrups were inspected for the presence of polysaccharides.

Moving-boundary electrophoresis has provided in recent years a technique for establishing the degree of homogeneity of a polysaccharide preparation. Fractionation procedures can be evaluated by electrophoretic examination of the fractions, and a suitable electrophoretically homogeneous fraction chosen for characterization. Northcote (1954) has established that borate ion is necessary to give a charge to neutral polysaccharides, while Bernier (1958) has examined several polysaccharides from different soils with this method. Lewis and Smith (1957) have also shown that electrophoresis on glass-fibre paper in strong alkali could indicate a heterogeneity that was not suspected from previous results.

The isolation of individual polysaccharide types from mixtures has been carried out successfully by fractional precipitation with ethanol at a convenient pH (Whistler and Lauterbach 1958). The shape of the fractionation curve has also been used to indicate the homogeneity of the preparations (Whistler and Be Miller 1956; Whistler and Kirby 1956). Optical rotation and identification of the component sugars in the fractions also give some indication of the efficiency of the fractionation procedure (Adams 1957).

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## II. Experimental

### (a) *Isolation of Crude Polysaccharide Preparations from Syrups*

Syrups were obtained from various sugar mills in Australia and Fiji throughout the crushing season and stored in the cold until required.

The syrup was diluted with water until its concentration was 60 per cent. (w/v) and 4 volumes of ethanol were added. The precipitate was centrifuged off, the water-soluble fraction dissolved in water, and the solution dialysed for 48 hr in the cold against five changes of water. The dialysis residue was then passed over a cellulosic anion exchanger (see below) to remove protein and most of the colour.

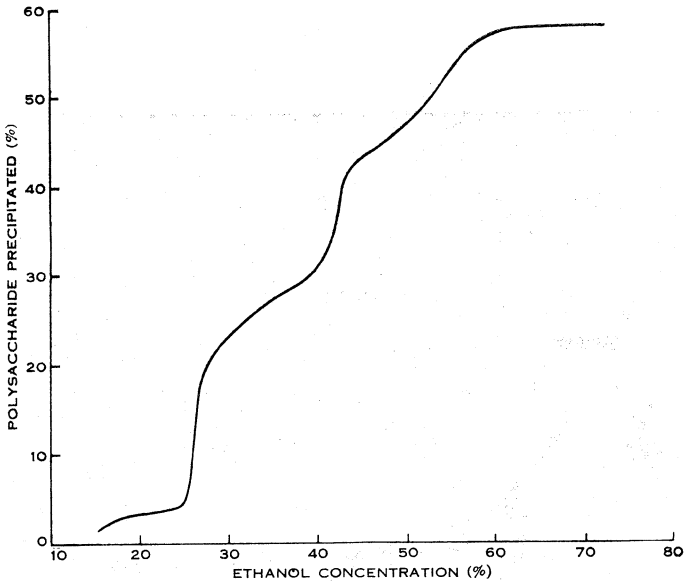


Fig. 1.—Fractional precipitation of a crude polysaccharide preparation from a 2 per cent. aqueous solution at pH 4·5.

The column was washed with water, and the effluent and washings were concentrated at 35–40°C under vacuum until the concentration of dissolved material was approximately 3 per cent. The crude polysaccharide material was then obtained by adjusting the pH to 4·5, precipitating with 4 volumes of ethanol, and drying the precipitate *in vacuo*.

### (b) *Cellulosic Anion Exchanger*

This was prepared from ethyl cellulose (Hercules Powder Co., Delaware, U.S.A.) by a method based on that of Peterson and Sober (1956) and adapted for this Department by B. Cortis-Jones (personal communication).

### (c) *Fractionation of the Crude Polysaccharide Preparations*

The dried material was broken up and dissolved in water to 1–2 per cent. concentration, and any insoluble material removed by centrifuging. The pH was adjusted to 4·5 with acetic acid, and ethanol added slowly with continual stirring.

Fractions were collected by centrifuging when sufficient material had precipitated, and were dried at 35–40°C under vacuum for several hours.

### (*d*) *Moving-boundary Electrophoresis*

This was carried out with a Perkin-Elmer Model 38A apparatus according to the method of Northcote (1954).

### (*e*) *Paper Electrophoresis*

This was carried out on glass-fibre paper according to Briggs, Garner, and Smith (1956) and carbohydrate zones detected with a solution of *p*-anisidine sulphate in ethanol used as a dip (1 g *p*-anisidine+2 ml concd. sulphuric acid in 100 ml ethanol—see Fuller and Northcote 1956).

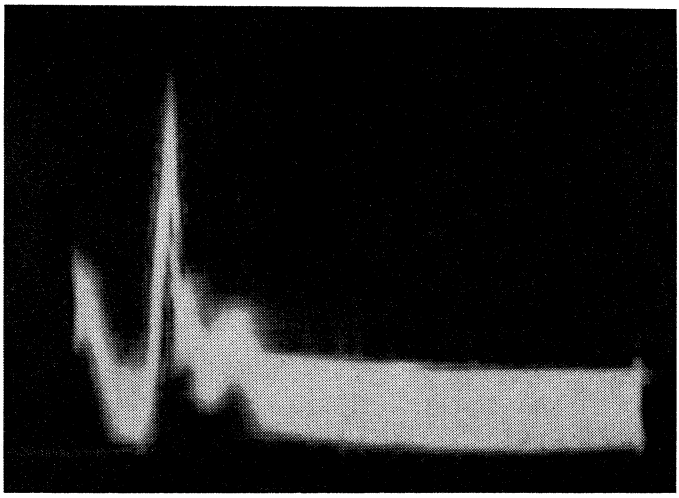

Fig. 2.—Electrophoretic pattern of a crude polysaccharide preparation from sugar syrup: borate buffer (pH 9·3, 10 mA, 1 per cent. polysaccharide); ascending boundary exposed at 50 min.

### (*f*) *Polysaccharide Hydrolysis and Paper Chromatography of Sugars*

Samples (20 mg) were heated at 100°C for 8 hr in 2N sulphuric acid under reflux. The solutions were neutralized with saturated barium hydroxide, centrifuged, and the supernatant dried at 35–40°C under vacuum. The residue was dissolved in 2 ml water, and the appropriate amount (10–50 $\mu$l) applied to the paper chromatogram with an "Agla" micrometer syringe. Mild hydrolysis to liberate arabinose was carried out in 0·1N oxalic acid at 100°C for ½–1 hr and the sugars recovered similarly.

The chromatograms were run in a benzene–butanol–pyridine–water solvent (1 : 5 : 3 : 3 v/v) overnight by descending chromatography (Hathway and Seakins 1958). Other solvents used were ethyl acetate–pyridine–water (8 : 2 : 1 and 2 : 1 : 2 v/v—Whistler and Kirby 1956). The sugars were most conveniently located by

detection with alkaline silver nitrate, adapting the method of Trevelyan, Procter, and Harrison (1950). The chromatogram was dipped in the silver nitrate solution, but the alkaline spray was replaced by dipping in a solution prepared by diluting 50 ml of 40 per cent. sodium hydroxide to 1 l. with ethanol. The chromatogram was then air dried, washed in a fixing solution (10 per cent. sodium thiosulphate + 1·5 per cent. sodium metabisulphite + 15 ml acetic acid per litre of solution) until the background colour disappeared, and finally washed for 1 hr in running water. After this treatment the chromatograms could be preserved for a considerable time. The sugars could also be detected by spraying with *p*-anisidine hydrochloride and heating (Hough, Jones, and Wadman 1950).

TABLE 1

FRACTIONAL PRECIPITATION OF A 1 PER CENT. AQUEOUS POLYSACCHARIDE MIXTURE

| Fraction | Electrophoretic Mobility* ($cm^2\,sec^{-1}\,volt^{-1}$) | Ethanol Concn. (%) | Optical Rotation $[\alpha]_{Hg}^{20}$ | Sugars Detected† |
|---|---|---|---|---|
| 1 | $1{\cdot}4\times10^{-5}$ | 41·6 | +198° | Galactose (m), glucose (M), arabinose (m), xylose (t), rhamnose (t) |
| 2 | | 46·1 | +176° | Galactose (m), glucose (M), arabinose (m), xylose (m), rhamnose (t) |
| 3 | | 48·1 | —‡ | |
| 4 | | 50·0 | —‡ | |
| 5 | | 53·3 | +140° | |
| 6 | | 54·8 | + 93° | |
| 7 | | 57·6 | + 57° | Galactose, glucose, arabinose, xylose, rhamnose (t) |
| 8 | $2{\cdot}5\times10^{-5}$ | 60·0 | —‡ | |

* pH 9·3 borate buffer.

† M = major, m = minor, t = trace.

‡ Insufficient material.

### (*g*) *Optical Rotation*

This was measured for a 1 per cent. aqueous solution on an ETL-NPL automatic polarimeter, type 143A, using a mercury lamp. This instrument enabled measurements to be made on small amounts of material.

### (*h*) *Viscosity*

Measurements were made on syrups brought to a standard concentration (73 per cent. solids by weight) at 60°C in a Höppler rheoviscometer. The syrup viscosity was compared with the viscosity of a sucrose solution under the same conditions, and used as a preliminary guide in the selection of syrups with various polysaccharide contents.

### (*i*) *Dextrans*

Dextran was prepared from *Leuconostoc mesenteroides*, and a sample of cane dextran was prepared from stale sugar-cane (Nicholson and Lilienthal 1959). The dry solids were obtained by ethanol precipitation and vacuum drying at 40°C.

## III. Results and Discussion

Precipitation with ethanol from a diluted syrup gave a material with a high ash content. This was reduced to a suitably low figure (1–5 per cent.) by subsequent solution and dialysis. After preparations from several syrups had been investigated, a pattern began to emerge connecting the viscosity of the original

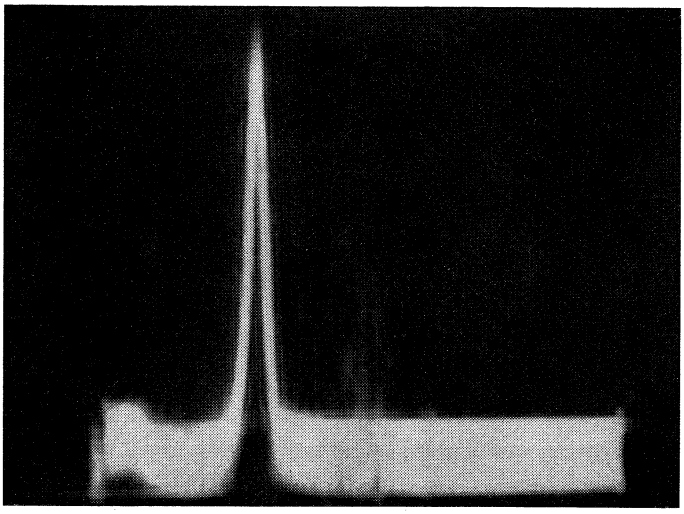

Fig. 3.—Electrophoretic pattern of a polyglucose isolated from the polysaccharide mixture in Figure 2: borate buffer (pH 9·3, 10 mA, 0·75 per cent. polysaccharide); ascending boundary exposed at 120 min.

syrup, the number of components in the crude polysaccharide that were resolved by moving-boundary electrophoresis, and the relative amounts of sugars that appeared on chromatograms of the hydrolysates.

The material from low-viscosity syrups gave chromatographic evidence for xylose, arabinose, glucose, and galactose in rather similar proportions, as well as a trace of rhamnose. The electrophoretic pattern indicated the presence of one main component. The appearance of another component with a slower but sharper boundary was associated with syrups of a slightly higher viscosity.

When the crude polysaccharide material from high-viscosity syrups was examined, a very strong glucose spot appeared on chromatograms of the hydrolysate, as well as the other sugar spots obtained before. The increase in glucose content coincided with the appearance on the electrophoretic patterns of a very

strong peak, corresponding in mobility to the peak which had begun to appear in the preparations from medium-range viscosity syrups. The amount of this component (as judged by electrophoresis) and the amount of glucose found on hydrolysate chromatograms increased with the viscosity of the original syrup.

These results indicated that there were two polysaccharide components that could occur in the syrups. One of these appeared to be related to increases in viscosity noticed during the season. The absence of any blue colour when crude polysaccharide preparations were tested with a dilute iodine solution suggested the absence of starch; nor was there any positive test for uronic acids (Dische 1947).

TABLE 2

PROPERTIES OF ISOLATED POLYSACCHARIDES AND DEXTRANS

| Compound | Optical Rotation* $[\alpha]^{20}_{Hg}$ | Electrophoretic Mobility† ($cm^2\ sec^{-1}\ volt^{-1}$) | Sugars Detected‡ |
|---|---|---|---|
| Syrup dextran | +198° to +240° | $1\cdot2$–$1\cdot5\times10^{-5}$ | Glucose (M), arabinose (t) |
| Stale cane dextran | +228° | $2\cdot6\times10^{-5}$ | Glucose |
| Dextran from *L. mesenteroides* § | +251° | $1\cdot7\times10^{-5}$ | Glucose |
| Syrup hemicellulose | +57° to +98° | $2\cdot4$–$2\cdot5\times10^{-5}$ | Galactose, glucose, arabinose, xylose (m), rhamnose (t) |

* 1 per cent. aqueous solution.

† Measured in 0·05M sodium borate solution (Northcote 1954), $I = 10$ mA, using ascending boundary.

‡ M = major, m = minor, t = trace.

§ This material showed a minor fast electrophoretic component.

The crude material was soluble in 1N sodium hydroxide, and gave very little precipitate with Fehling's solution (Chanda *et al.* 1950), or with cetyl trimethylammonium bromide and sodium borate (Barker, Stacey, and Zweifel 1957). After mild hydrolysis with 0·1N oxalic acid, arabinose only could be detected in chromatograms.

The crude polysaccharide material was fractionally precipitated with ethanol at pH 4·5. This has been suggested as an analytical and a preparative technique by Whistler and Lauterbach (1958). Figure 1 shows the shape of a typical fractionation curve from a crude material. Figure 2 is the electrophoretic pattern of this same polysaccharide mixture. Fractionation at pH 4–5 assisted in the removal of most of the colour. Table 1 shows some characteristics of fractions collected from a typical preparation.

The shape of the fractionation curve indicated that the two main polysaccharides could be separated by this precipitation method. Examination of the

appropriate fractions showed that both polysaccharides could be obtained electrophoretically homogeneous. The fraction which was collected at lower ethanol concentrations (Fig. 3) was essentially a polyglucose, although several precipitations with ethanol did not remove final traces of arabinose from chromatograms of the hydrolysate. The high positive rotation suggests it is a dextran type, although it has a different mobility from the two other dextrans inspected (Table 2). The other polysaccharide is a heteroglycan of the hemicellulose class. Some properties of the two polysaccharides, together with the two dextrans inspected, are included in Table 2. The separated polysaccharides also showed different rates of movement when subjected to electrophoresis using glass-fibre paper.

The presence of two main polysaccharide constituents of sugar syrups has thus been established, one a dextran and the other a hemicellulose. The hemicellulose has been present in all syrups examined and is probably a cell-wall constituent. Nicholson and Lilienthal (1959) showed that an abnormal dextran could form in sugar-cane during a period of delay before the cane is crushed. The dextran which has been found in sugar syrups could well be caused by abnormal metabolism or infection of the cane, if any delay occurs between the burning and the crushing of the cane. This dextran contributes to the abnormally high viscosities observed in some syrups.

## IV. Acknowledgments

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